UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,192,927 B2
APPLICATION NO. : 10/776224
DATED : March 20, 2007
INVENTOR(S) : Gustav Guadernack et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

COLUMNS 35-40

Delete column 35, line 2, to column 40, line 64, and replace with attached page 52, line 8, to page 63, line 38, of the original specification as filed.

Signed and Sealed this

Seventh Day of September, 2010

David J. Kappos

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result in an overlapping peptide. This has now been proven for CD4 T cells by the results in Fig. 8-11. These results have the following implication:

1) The results in Figure 8 prove that the mutated form of TGFβRII Receptor which occurs in a high proportion of cancer patients with defects in their mismatch repair machinery is a tumour specific antigen.

2) The antigen specificity of the infiltrating T cells commonly observed in colorectal cancer are generally not known. The results in figure 8 demonstrate that one component of the T cells constituting the population of tumour infiltrating lymphocytes in this patients tumour is specific for a frameshift mutation, demonstrating that TGFβRII frameshift peptides are immunogenic *in vivo,* occasionally giving rise to spontaneous T cell activation.

3) It follows from this observation that processing of the non-functional form of the TGFβRII Receptor that is formed by the common frameshift mutation is processed. This processing may take place either in the tumour cell as part of natural breakdown of the aberrant protein, or after the tumour cell itself or a released form of the receptor has been taken up by a professional APC or both.

4) The results in Figure 8 also indicate that the peptide with seq. id. no. 17 is capable of binding to an HLA class II molecule, since pulsing of APC with this peptide results in a specific proliferative response against the peptide, and since CD4 T cell responses always are class II restricted. That this is the case is demonstrated by the results of the experiment shown in Figure 9. Here it is shown that the specific response against the peptide with seq. id. no. 17 is completely blocked by an antibody to HLA-DR, but not with antibodies to the two other HLA class II molecules, HLA-DQ and -DP. Furthermore, by using a panel of standard homozygous Epstein Barr Virus (EBV) transformed B Cell Lines (BCL) covering the relevant HLA class II molecules present on the patients own APC, we were able to identify the class II molecule responsible for presentation of the peptide with seq. id. no. 17 to TLC IMT8 and IMT9 as being HLA-DR 14. Together these findings fit extremely well with the immunohistological observations made in parallel sections taken from the same tumour biopsy, where we could show that activated CD4+ T cells were abundant in the proximity of tumour cells that had been induced to express HLA-DR molecules. The results in Figure 11 demonstrate that these T cell clones are capable of mounting a proliferative response over a range of peptide doses and that the responses are dose dependent.

5) Since these T cell clones were obtained by cloning T cells isolated from a tumour biopsy, another implication of our finding is that activated T cells specific for the peptide with seq. id. no. 17 are capable of homing to the tumour tissue after activation.

6) Since the peptide with seq. id. no. 17 is a tumour specific antigen, and since frameshift mutations giving rise to this peptide or peptides with overlapping sequences are commonly found in cancers with defects in enzymes that are part of the mismatch repair machinery, this peptide may be used as a vaccine to elicit T cell response in cancer patients or patients at high risk for developing cancer. Such T cell responses may potentially influence the growth of an existing tumour or prohibit regrowth of tumour after surgery and other forms of treatment or be given to patients with an inheritable form of cancer where a defect mismatch enzyme is detected or suspected and that have a high chance of developing a cancer where this precise mismatch repair mutation will occur.

Synthesis

The peptides were synthesised by using continuous flow solid phase peptide synthesis. N-a-Fmoc-amino acids with appropriate side chain protection were used. The Fmoc-amino acids were activated for coupling as pentafluorophenyl esters or by using either TBTU or diisopropyl carbodiimide activation prior to coupling. 20% piperidine in DMF was used for selective removal of Fmoc after each coupling. Cleavage from the resin and final removal of side chain protection was performed by 95% TFA containing appropriate scavengers. The peptides were purified and analysed by reversed phase (CI8) HPLC. The identity of the peptides was confirmed by using electro-spray mass spectroscopy (Finnigan mat SSQ710).

The peptides used for *in vitro* studies of T cell stimulation were synthesised by this method.

Several other well known methods can be applied by a person skilled in the art to synthesise the peptides.

Examples of the method for determining new frameshift mutation peptides.

In this Example, the BAX gene is used to illustrate the principle.

In each of the steps listed below, the 1st line is the gene sequence and 2nd line is amino acid sequence.

In the steps 2-5, the outlined sequences represent the mutant part of the protein.

Step one:

Normal BAX.

```
ATG GGG GGG GAG GCA CCC GAG CTG GCC CTG GAC CCG GTG .....
    M   G   G   E   A   P   E   L   A   L   D   P   V ...
```

Step two:

1G deleted from gene sequence.

```
ATG GGG GGG AGG CAC CCG AGC TGG CCC TGG ACC CGG TGC CTC
    M   G   G   R   H   P   S   W   P   W   T   R   C  L

AGG ATG CGT CCA CCA AGA AGC TGA
 R   M   R   P   P   R   S  stop
```

Step three:

2G deleted from gene sequence.

```
ATG GGG  GGA GGC ACC CGA GCT GGC CCT GGA CCC GGT GCC
 M   G    G   G   T   R   A   G   P   G   P   G   A

TCA GGA TGC GTC CAC CAA GAA GCT GAG CGA GTG TCT CAA GCG
 S   G   C   V   H   Q   E   A   E   R   V   S   Q   A

CAT CGG GGA CGA ACT GGA CAG TAA
 H   R   G   R   T   G   Q  stop
```

Step four:

1G inserted in gene sequence.

ATG GGG GGG ***GGA GGC ACC CGA GCT GGC CCT GGA CCC GGT GCC***
M G G **G G T R A G P G P G A**

TCA GGA TGC GTC CAC CAA GAA GCT GAG CGA GTG TCT CAA GCG
S G C V H Q E A E R V S Q A

CAT CGG GGA CGA ACT GGA CAG *TAA*
H R G R T G Q stop

Step five:

2G inserted in gene sequence.

ATG GGG GGG GGG ***AGG*** *CAC CCG AGC TGG CCC TGG ACC CGG TGC*
M G G G **R H P S W P W T R C**

CTC AGG ATG CGT CCA CCA AGA AGC TGA
L R M R P P R S stop

In the next Example, the TGFβRII gene is used to illustrate the principle.

In each of the steps listed below, the 1st line is the gene sequence and 2nd line is amino acid sequence.
In the steps 2-5, the outlined sequences represent the mutant part of the protein.

Step one:

Normal TGFβRII.

GAA AAA AAA AAG CCT GGT GAG ACT TTC TTC ATG TGT TCC....
E K K K P G E T F F M C S...

Step two:

1A deleted from gene sequence.

GAA AAA AAA AGC CTG GTG AGA CTT TCT TCA TGT GTT CCT GTA
E K K S L V R L S S C V P V

GCT CTG ATG AGT GCA ATG ACA ACA TCA TCT TCT CAG AAG AAT
A L M S A M T T S S S Q K N

ATA ACA CCA GCA ATC CTG ACT TGT TGC TAG
I T P A I L T C C stop

Step three:

2A deleted from gene sequence.

GAA AAA AAA GCC TGG TGA
E K K A W stop

Step four:

1A inserted in gene sequence.

GAA AAA AAA AAA GCC TGG TGA
E K K K A W stop

Step five:

2A inserted in gene sequence.

GAA AAA AAA AAA AGC CTG GTG AGA CTT TCT TCA TGT GTT CCT
E K K K S L V R L S S C V P

GTA GCT CTG ATG AGT GCA ATG ACA ACA TCA TCT TCT CAG AAG
V A L M S A M T T S S S Q K

AAT ATA ACA CCA GCA ATC CTG ACT TGT TGC TAG
N I T P A I L T C C stop

Thus the peptides of the invention may be used in a method for the treatment of cancers with cancer cells harbouring genes with frameshift mutations, which treatment comprises administering at least one peptide of the present invention *in vivo* or *ex vivo* to a human patient in need of such treatment.

In another embodiment the peptides of the invention may be used to vaccinate a human being disposed for cancers with cancer cells harbouring genes with frameshift mutations, by administering at least one peptide of the present invention to said human being.

It is further considered to be an advantage to administer to a human individual a mixture of the peptides of this invention, whereby each of the peptides of the invention can bind to different types of HLA class I and/or class II molecules of the individual.

It is further anticipated that the power of an anticancer vaccine or peptide drug as disclosed in the above mentioned PCT/NO92/00032 application, can be greatly enhanced if the peptides of the present invention were included. Thus in another embodiment of the present invention peptides of the present invention are administered together with, either simultaneously or in optional sequence, with the peptides disclosed in PCT/NO92/00032.

It is considered that the peptides may be administered together, either simultaneously or separately, with compounds such as cytokines and/or growth factors, i.e. interleukin-2 (IL-2) , interleukin-12 (IL-12), granulocyte macrophage colony stimulating factor (GM-CSF), Flt-3 ligand or the like in order to strengthen the immune response as known in the art.

The peptides according to the present invention can be used in a vaccine or a therapeutical composition either alone or in combination with other materials, such as for instance standard adjuvants or in the form of a lipopeptide conjugate which as known in the art can induce high-affinity cytotoxic T lymphocytes, (K. Deres, Nature, Vol. 342, (Nov. 1989)).

The peptides according to the present invention may be useful to include in either a peptide or recombinant fragment based vaccine.

The peptides according to the present invention can be included in pharmaceutical compositions or in vaccines together with usual additives, diluents, stabilisers or the like as known in the art.

According to this invention, a pharmaceutical composition or vaccine may include the peptides alone or in combination with at least one pharmaceutically acceptable carrier or diluent.

Further a vaccine or therapeutical composition can comprise a selection of peptides which are fragments of the mutant proteins arising from insertion or deletion of bases in a repeat sequence of the gene.

Further a vaccine composition can comprise at least one peptide selected for one cancer, which vaccine would be administered to a person carrying a genetic disposition for this particular cancer.

Further a vaccine composition can comprise at least one peptide selected for one cancer, which vaccine would be administered to a person belonging to a high risk group for this particular cancer.

The cancer vaccine according to this invention may further be administered to the population in general for example as a mixture of peptides giving rise to T cell immunity against various common cancers connected with frameshift mutation genes.

The peptides according to this invention may be administered as single peptides or as a mixture of peptides. Alternatively the peptides may be covalently linked with each other to form larger polypeptides or even cyclic polypeptides.

A cancer therapy according to the present invention may be administered both in vivo or ex vivo having as the main goal the raising of specific T cell lines or clones against the mutant gene product associated with the cancer type with which the patient is afflicted.

Further, the frameshift mutant peptides of this invention may be administered to a patient by various routes including but not limited to subcutaneous, intramuscular, intradermal, intraperitoneal, intravenous or the like. In one embodiment the peptides of this invention are administered intradermally. The peptides may be administered at single or multiple injection sites to a patient in a therapeutically or prophylactically effective amount.

The peptides of this invention may be administered only once or alternatively several times, for instance once a week over a period of 1-2 months with a repeated sequence later all according to the need of the patient being treated.

The peptides of this invention can be administered in an amount in the range of 1 microgram (1 μg) to 1 gram (1g) to an average human patient or individual to be vaccinated. It is preferred to use a smaller dose in the range of 1 microgram (1 μg) to 1 milligram (1 mg) for each administration.

The invention further encompasses DNA sequences which encodes a frameshift mutation peptide.

The invention additionally encompasses isolated DNA sequences comprising a DNA sequence encoding at least one frameshift mutant peptide, and administration of such isolated DNA sequences as a vaccine for treatment or prophylaxis of cancers associated with frameshift mutations in the genes.

The peptides according to this invention may be administered to an individual in the form of DNA vaccines. The DNA encoding these peptides may be in the form of cloned plasmid DNA or synthetic oligonucleotide. The DNA may be delivered together with cytokines, such as IL-2, and/or other co-stimulatory molecules. The cytokines and/or co-stimulatory molecules may themselves be delivered in the form of plasmid or oligonucleotide DNA. The response to a DNA vaccine has been shown to be increased by the presence of immunostimulatory DNA sequences (ISS). These can take the form of hexameric motifs containing methylated CpG, according to the formula: 5'-purine-purine-CG-pyrimidine-pyrimidine-3'. Our DNA vaccines may therefore incorporate these or other ISS, in the DNA encoding the peptides, in the DNA encoding the cytokine or other co-stimulatory molecules, or in both. A review of the advantages of DNA vaccination is provided by Tighe et al (1998, *Immunology Today*, 19(2), 89-97).

In one embodiment, the DNA sequence encoding the mutant BAX peptides comprises:

Normal BAX.

ATG GGG GGG GAG GCA CCC GAG CTG GCC CTG GAC CCG GTG .....

1G deleted from BAX gene sequence.

*ATG GGG GGG **AGG CAC CCG AGC TGG CCC TGG ACC CGG TGC CTC***

***AGG ATG CGT CCA CCA AGA AGC** TGA*

2G deleted from BAX gene sequence.

*ATG GGG **GGA GGC ACC CGA GCT GGC CCT GGA CCC GGT GCC***

TCA GGA TGC GTC CAC CAA GAA GCT GAG CGA GTG TCT CAA GCG

***CAT CGG GGA CGA ACT GGA CAG** TAA*

1G inserted in BAX gene sequence.

*ATG GGG GGG **GGA GGC ACC CGA GCT GGC CCT GGA CCC GGT GCC***

TCA GGA TGC GTC CAC CAA GAA GCT GAG CGA GTG TCT CAA GCG

***CAT CGG GGA CGA ACT GGA CAG** TAA*

2G inserted in BAX gene sequence.

*ATG GGG GGG GGG **AGG** CAC CCG AGC TGG CCC TGG ACC CGG TGC*

CTC AGG ATG CGT CCA CCA AGA AGC TGA

In a second embodiment, the DNA sequence encoding the mutant TGFβRII peptides comprises:

Normal TGFβRII gene.

GAA AAA AAA AAG CCT GGT GAG ACT TTC TTC ATG TGT TCC....

1A deleted from TGFβRII gene sequence.

GAA AAA AAA AGC CTG GTG AGA CTT TCT TCA TGT GTT CCT GTA

GCT CTG ATG AGT GCA ATG ACA ACA TCA TCT TCT CAG AAG AAT

ATA ACA CCA GCA ATC CTG ACT TGT TGC TAG

2A deleted from TGFβRII gene sequence.

GAA AAA AAA GCC TGG TGA

1A inserted in TGFβRII gene sequence.

GAA AAA AAA AAA GCC TGG TGA

2A inserted in TGFβRII gene sequence.

GAA AAA AAA AAA AGC CTG GTG AGA CTT TCT TCA TGT GTT CCT

GTA GCT CTG ATG AGT GCA ATG ACA ACA TCA TCT TCT CAG AAG

AAT ATA ACA CCA GCA ATC CTG ACT TGT TGC TAG

The invention further encompasses vectors and plasmids comprising a DNA sequence encoding a frameshift mutant peptide. The vectors include, but are not limited to *E. Coli* plasmid, a Listeria vector and recombinant viral vectors. Recombinant viral vectors include, but are not limited to orthopox virus, canary virus, capripox virus, suipox virus, vaccinia, baculovirus, human adenovirus,